A Preliminary investigation of potential antimicrobial activity of *Solenostemma Oleifolium*

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ABSTRACT

Four compounds (A, B, C, and D) had been extracted from the stems of a desert-plant, *Solenostemma Oleifolium*. Pending spectroscopic identification, these compounds were subjected to a preliminary screening for potential antimicrobial activity against, four selected genera of gram-positive and gram-negative bacteria by the tube dilution tecnique. Only compound C showed reasonable antibacterial properties against both gram positive and gram negative organisms. None of these compunds was active against *Pseudomonas aeruginosa*.

INTRODUCTION

Solenostemma is a monotypic genus distributed in the deserts of Arabia, Egypt, Libya (Fezzan), Chad, Sudan and Palestine. *S. oleifolium* (Nectoux), family Asclepiadeceae is an erect shrub reaching a height of 60-100 cm with many velvety pubescent branches from the base. The leaves are fleshy, subsessile, ovate-oblong to elliptic and have a velvet like pubescence. The umbel is axillary with a short peduncle. The calyx consists of five seprate sepals and the corolla of five white, separate petals, 7-10 mm broad with erect, oblong-linear lobes. The follicle is oblong to ovate, smooth and very hard (4). The leaves were formerly used to adulterate senns and are considered to be medicinally important in Libya and Chad where a decoction is used to treat neuralgia and sciatica. In Libya the plant is called by the local Arabic name of «Argel». A Hungarian report (5) on S. argel showed the presence of Kaempferol and steroidal glycosides while an Egyptian team (6) isolated two substances arbitrarily designated the names argeloside and argelin.The first compound was found in small amounts as a crystalline glycoside with a glucose moiety linked to a noncardenolide aglycone while the second is also crystalline but non-glycosidal in nature. No further details were supplied. Four 11 -B-hydroxylated ent-kaurene derivatives have been isolated from S. triste (2).

MATERIALS AND METHODS

The plants were collected in February fron Southern Libya and a specimen was lodged in University of Al-Fateh Herbarium. Air-dried powdered stems 450 grams were extracted in a Soxhlet with petroleum ether (60-80) for 24 hours. Four pure compounds were isolated (Scheme 1). Minimal inhibitory concentration (MIC) of these compounds

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were determined by the tube dilution technique using nutrient broth, pH 7.4 as a basal medium (1,3). Four organisms were selected for screening, namely *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and a *Proteus species*. Each of the screened compounds was dissolved in dimethylformamide (DMF) in a concentration of 1 mg/ml and these solutions were doubly diluted with sterile broth to obtain the appropriate concentration. To all the tubes, an appropriate concentration of each test organism was added in each group of tests. Tubes of uninoculated medium with and without the compounds were included as controls. A third control tube containing inoculated medium was also included to ascertain growth ability in the medium. All the tubes were incubated at 37°C for 18 - 24 hours.

RESULTS AND DISCUSSION

From the extraction procedure (Scheme 1), four pure compounds were isolated:

Compound A: Crystallised from acetone m.p. 55°

Compound B: Crystallised from methanol m.p. 104°

Compound C: From the saponifiable fraction and crystallised from methanol m.p. 78°

Compound D: From the chloroform extract, colourless crystals from methanol m.p. 84°.

All m.ps are uncorrected and the compounds are awaiting spectroscopic data for identification.

Standard extraction procedures followed by column and thin layer chromatography allowed the isolation of four pure compounds from the stems of *Solenostemma oleifolium*, a rarer member of the desert flora of Southern Libya. Pending spectroscopic identification, the four compounds were subjected to a preliminary antimicrobial screening procedure. The serial tube dilution method was chosen as a useful, simple technique, relatively easily controlled and requiring no sophisticated equipments.

The test organisms were chosen to represent typical gram-positive and gram-negative bacteria; the latter included. *P. aeurginosa* as a higly resistant species. The need for the use of small inocula has long been recognised due to the presence of some organisms of greater resistance in larger inocula. Further, the potential antimicrobial compound should be in excess of the amount adsorbed escpecially when the concentration is near the minimum that is effective. A pH of 7.4 was used for the medium to minimise inhibi-

Scheme 1 — Extraction of the stems of *S. oleifolium*.

Air dried stems (450 gm) Extracted with P. ether 60-80° (Soxhlet, 24 hours)

MARC
Extracted with chloroform
(Soxhlet, 12 hrs.)

Extract yellow solutions evaporated under reduced pressure

Extract yellow solution evaporated under-reduced pressure

Yellow-known oily residue (5.6g, 1,44%) saponified, 10% ethanolic KOH, One hour

Yellowish oil crystallized from MeOH
Colourless crystals
m.p. 84 (D)
(6 g)

insoluble residue

Saponifiable fraction
white solid
(0.75 g)
recrystallized from MeOH
compound C m.p. 78°

unsaponifiable fraction
column chromatography
Silicagel (80 × 2 cm).

Elution by 1 – Toluene
Compound A
yellow platet crystals 4.1 gm
m.p. 55° 2 – Toluene/methanol 9:1 Compound B Clourless crystals (2.2 gm) m.p. 105°

tion of bacterial growth due to increasing acidity of the nutrient solution. The effect of pH on the potential antibacterial activity of compounds was unknown but it could be influenced by acidity or alkalinity of the medium.

As can be observed from Table 1, the negative results of all the unknown compounds against *P. aeruginosa* and *Proteus* are not entirely unexpected. The results showed some variation in the sensitivities of *S. Aureus* and *E. Coli* to the four new compounds, compound C being the most effective against both species and the only one to show activity against *Proteus* spp.

Table 1 — Minimal inhibitory concentration of four potential antibacterial substances[a].

	S. aureus	*E. coli*	*P. aeruginosa*	*Proteus spp*
Penicillin G	0.016	14	500	Not tested
Compound A	250	250	—	—
Compound B	500	—	—	—
Compound C	125	250	—	250
Compound w2	250	500	—	—
Oxytetracycline	0.13	0.5	8	Not tested

[a] Units : mcg/ml

— Negative result.

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نشاط نبات الحرجل المضاد للميكروبات

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المستخلص

تم استخلاص أربعة مركبات (أ، ب، جـ، د) من سيقان نبات الحرجل الصحراوى.

قبل التحليل الاسبكتروسكوبى تمت دراسة التأثير المثبط لهذه المركبات على اربعة ميكروبات مختارة من موجب وسالب الجرام باستعمال طريقة التخفيف بالانابيب.

المركب جـ فقط أظهر تأثيرا مثبطا على معظم الميكروبات وفى نفس الوقت أظهرت جميع المركبات عدم تأثيرها على ميكروب *Pseudomonas aeruginosa* .